REMARKS

Entry of the foregoing prior to the initial office action on the merits is respectfully requested. Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version With Markings to Show Changes Made.

Applicants submit that the above amendments are made to correct inconsistencies in the specification as filed and the Sequence Listing submitted herewith, and that written support is provided in the specification as filed for all amendments. In particular, the amendment of SEQ. ID. No. 5 and SEQ. ID. No. 6, wherein "X" and "Y," respectively, are changed to "N" is supported in the specification by the paragraph beginning at page 39, line 27, including Table 1, in combination with the requirements of 37 C.F.R. § 1.821. As described in the noted paragraph and shown in Table 1, SEQ. ID. No. 5 and SEQ. ID. No. 6 are synthetic nucleotide substrates used to test the cleavage properties of *Tma* endonuclease V. Because every possible base substitution may be made at the positions held by "X" and "Y," it is clear that both "X" and "Y" can be a, t, c, or g. Therefore, the "X" and "Y" have been renamed "N" so as to be in accord with 37 C.F.R. § 1.821, which requires the use of the World Intellectual Property Organization (WIPO) convention for sequence identifiers. WIPO Std. ST.25, Appendix 2, Table 1, provides that the single base identifier for a nucleotide sequence that may be "a or g or t or c" is "N." For all these reasons, applicants submit the amendment of SEQ. ID. No. 5 and SEQ. ID. No. 6 merely corrects this inconsistency between the specification and the Sequence Listing accompanying this paper.

Next, the identification of a replacement "SEQ. ID. No. 50" in the specification and the Sequence Listing is supported in the specification as filed by Figure 19 and the paragraph beginning on page 74, line 11, which describes Figure 19. Figure 19 shows a primary amino acid sequence alignment among 13 identified and putative Endo V enzymes from thermophilic and mesophilic archeabacteria and eubacteria described in the present invention beginning at page 74, line 11, and shows the majority amino acid sequence of those 13 amino acids in the top line of Figure 19, labeled therein as the "majority" sequence. This amino acid sequence was not given a sequence identifier in the specification as filed, therefore, that is corrected by the above amendment and the inclusion of this sequence as SEQ. ID. No. 50 in the Sequence Listing accompanying this paper.

Written support for changing SEQ. ID. No. 50 to SEQ. ID. No. 1, and SEQ. ID. No. 51 to SEQ. ID. No. 2, is found at page 12, lines 10-18, in combination with Figure 3A. As described on page 12, Figure 3A shows an assay design where the top strand (SEQ.

ID. No. 1) is Fam labeled and the bottom strand (SEQ. ID. No. 2) is Tet labeled. The positions of nucleotide changes at both strands ("I") are underlined in Figure 3A. SEQ. ID. No. 1 and SEQ. ID. No. 2 shown in Figure 3A are identical to SEQ. ID. No. 50 and SEQ. ID. No. 51, respectively, described and shown in the specification in the paragraph bridging pages 83-84. Therefore, these sequences were incorrectly named SEQ. ID. No. 50 and SEQ. ID. No. 51, as they had already been named SEQ. ID. No. 1 and SEQ. ID. No. 2 earlier in the specification.

Because written support is provided for the above amendments in the application as filed, applicants submit that no new matter is added herein.

Respectfully submitted,

Date: November 11, 2002

Michael L. Goldman
Registration No. 30,727

NIXON PEABODY LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603-1051
Telephone: (585) 263-1304
Facsimile: (585) 263-1600

Certificate of Mailing - 37 CFR 1.8(a)
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: U.S. Patent and Trademark Office P.O. BOX 2327 Arlington, VA 22202, on the date below.
Date 11/12/02 | Wendy L. Barry

Appendix A

Version With Markings to Show Changes Made

In reference to the amendments made herein to specification, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In The Specification :

Please replace the paragraph beginning on page 39, line 27 with the following paragraph:

Table 1 provides a summary of results with *Tma* endonuclease V cleavage of heteroduplexed synthetic substrates containing single base mismatches. Note that for every possible base change, there are two possible heteroduplexed products which may form: A↔G (A-C, G-T); C↔T (C-A, T-G); A↔C (A-G, C-T); G↔T (G-A, T-C); A↔T (A-A, T-T), and G↔C (G-G, C-C).

Table 1. Summary of *Tma* endonuclease V cleavage of heteroduplexed synthetic substrates containing single base mismatches.

Base change (Wt↔Mt)	A↔G	C↔T	A↔C	G↔T	A↔T	G↔C
Heteroduplex I: UpperStrand (Wt) BottomStrand(Mt)	A +++ ǀ C -	C + ǀ A ++	A ++ ǀ G +++	G ++ ǀ A ++	A +++ ǀ A +++	G +++ ǀ G +++
Heteroduplex II: UpperStrand (Mt) BottomStrand (Wt)	G ++ ǀ T ++	T ++ ǀ G +++	C + ǀ T ++	T ++ ǀ C -	T + ǀ T +	C - ǀ C -

Note:
UpperStrand:
5'-FAM-TA CCC CAG CGT CTG CGG TGT TGC GTN[X] AGT TGT CAT AGT TTG ATC CTC TAG TCT TGT TGC GGG TTCC-3' (SEQ. ID. No. 5)

BottomStrand:
3'- GGG GTC GCA GAC GCC ACA ACG **CAN[Y]** TCA ACA GTA TCA AAC TAG GAG ATC AGA ACA ACG CCC-TET-5' (SEQ. ID. No. 6)

Appendix A

Version With Markings to Show Changes Made

Cleavage symbols: (+++): high intensity cleavage. (++): intermediate intensity cleavage. (+): low intensity cleavage. (-) no cleavage

The cleavage of these heteroduplexed products are not always identical (i.e. compare A-C with C-A), and this reflects subtleties in the structure of the DNA as a consequence of neighboring sequence variation. Nevertheless, for each possible base change, signal is generated for at least one top strand and at least one bottom strand. Thus, the *Tma* EndoV enzyme should be able to recognize any possible single base mutation or polymorphism.

Please replace the paragraph beginning at page 74, line 11 with the following paragraph:

Residues that are involved directly in protein-substrate interactions have a strong tendency to be conserved among enzymes of the same family. Therefore, in a primary amino acid sequence alignment, highly conserved residues represent good candidates for mutagenesis. In order to identify positions in *Thermotoga maritima* Endo V suitable for mutagenesis, the ClustalW alignment algorithm with a PAM250 Residue Weight Table(Pairwise Alignment Parameters: Ktuple = 1, Gap Penalty = 3, Window = 5, and Diagonals Saved = 5; Multiple Alignment Parameters: Gap Penalty = 10 and Gap Length Penalty = 10) was used to perform a primary amino acid sequence alignment among 13 identified and putative Endo V enzymes from thermophilic and mesophilic archeabacteria and eubacteria (i.e. *Thermotoga maritima* (SEQ. ID. No. 37), *Pyrobaculum aerophilum* (SEQ. ID. No. 38), *Pyrococcus horikoshii* (SEQ. ID. No 39), *Pyrococcus abyssi* (SEQ. ID. No. 40), *Pyrococcus furiosus* (SEQ. ID. No. 41), *Archaeoglobus fulgidus* (SEQ. ID. No. 42), *Aeropyrum pernix* (SEQ. ID. No. 43), *Clostridium acetobutylicum* (SEQ. ID. No. 44), *Yersinia pestis* (SEQ. ID. No. 45), *Escherichia coli* (SEQ. ID. No. 46), *Bacillus subtilis* (SEQ. ID. No. 47), *Salmonella typhimurium* (SEQ. ID. No. 48), and *Streptomyces coelicolor* (SEQ. ID. No. 49)<u>, majority sequence, top line (SEQ. ID. No. 50)</u> (Figure 19)). Since the majority of enzymes utilized in the alignment are putative Endo V enzymes, the mismatch specificity of most of these enzymes is unknown. As a result, when utilizing the alignment to identify candidate residues in *Thermotoga maritima* Endo V, one can either assume that the homologous enzymes have similar or different specificities. If one assumes similar

Appendix A

Version With Markings to Show Changes Made

specificities, then residues that are highly conserved for the majority of Endo V provides candidates. Whereas if one assumes different specificities, then positions where there exists two sets of highly conserved residues represent candidates.

Please replace the paragraph beginning at page 83, line 29, with the following paragraph:

The fluorescence labeled deoxyoligonucleotide substrates were prepared as described (Huang, J., et al., <u>Biochemistry</u> 40(30):8738-8748 (2001), which is hereby incorporated by reference in its entirety). The sequence of a typical inosine substrate (SEQ. ID. Nos. [50-51] <u>1-2</u>, respectively) is as follows:

```
5'-Fam-TA CCC CAG CGT CTG CGG TGT TGC GT   I AGT TGT CAT AGT TTG ATC CTC TAG TCT TGT TGC GGG TTC C-3'
        3'- GGG GTC GCA GAC GCC ACA ACG CA   I TCA ACA GTA TCA AAC TAG GAG ATC AGA ACA ACG CCC-Tet-5'
```

A nick event at the top strand generates a 27 nt labeled product while that at the bottom strand generates a 38 nt labeled product. The cleavage reactions were performed at 65°C for 30 minute in a 20 μl reaction mixture containing 10 mM HEPES (pH 7.4), 1 mM DTT, 2% glycerol, 5 mM $MgCl_2$ unless otherwise specified, 10 nM DNA substrate, indicated amount of purified Tma endonuclease V protein. The reaction was terminated by adding an equal volume of GeneScan stop solution. The reaction mixtures were then heated at 94°C for 2 min and cooled on ice. Three microliter of samples were loaded onto a 10% GeneScan denaturing polyacrylamide gel (Perkin Elmer). Electrophoresis was conducted at 1500 voltage for 1 hr using an ABI 377 sequencer (Perkin Elmer). Cleavage products and remaining substrates quantified using the GeneScan analysis software versions 2.1 or 3.0.